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Targeting gut flora to prevent progression of hepatocellular carcinoma

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COMMENTARY ON:

Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, Caviglia JM, Khiabanian H, Adeyemi A, Bataller R, Lefkowitch JH, Bower M, Friedman R, Sartor RB, Rabadan R, Schwabe RF. Cancer Cell. 2012 Apr 17;21(4): 504-16. Copyright © 2012. Abstract reprinted with permission from Elsevier.

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Abstract. *Increased translocation of intestinal bacteria is a hallmark of chronic liver disease and contributes to hepatic inflammation and fibrosis. Here we tested the hypothesis that the intestinal microbiota and Toll-like receptors (TLRs) promote hepatocellular carcinoma (HCC), a long-term consequence of chronic liver injury, inflammation, and fibrosis. Hepatocarcinogenesis in chronically injured livers depended on the intestinal microbiota and TLR4 activation in non-bone-marrow-derived resident liver cells. TLR4 and the intestinal microbiota were not required for HCC initiation but for HCC promotion, mediating increased proliferation, expression of the hepatomitogen epiregulin, and prevention of apoptosis. Gut sterilization restricted to late stages of hepatocarcinogenesis reduced HCC, suggesting that the intestinal microbiota and TLR4 represent therapeutic targets for HCC prevention in advanced liver disease.*

Chronic inflammation of the liver is a well-recognized risk factor for carcinogenesis, 80% of all cases of hepatocellular carcinoma (HCC) being associated with cirrhosis or fibrosis, diseases characterized by persistent cycles of liver injury, inflammation, and compensatory hepatocyte proliferation. Accumulating evidence supports the idea that persistent inflammation leads to HCC. In recent years, a number of studies have been dedicated to the characterization of signal transducing mediators that connect liver inflammation to carcinogenesis [1]. Recent studies have focused on the possible pro-inflammatory pro-tumor role of lipopolysaccharide (LPS), a pathogen-associated molecular pattern (PAMP) of the gut-liver axis. Indeed, high levels of LPS occur in patients with cirrhosis because of an increase in intestinal mucosal permeability and bacterial translocation (for review [2]). This activates the NF-κB pathway, produces proinflammatory cytokines (TNF-α, IL-6, and IL-1) and leads to liver inflammatory and oxidative damage. As LPS acts as a potent ligand for activation of the innate immunity Toll like receptor 4 (TLR4), experimental approaches have been based on alterations of intestinal LPS balance or TLR4 activity.

A first comprehensive study of the role of gut-derived LPS in hepatocarcinogenesis was published by Yu *et al.* in 2010 [3]. In that study, HCC was induced by diethylnitrosamine (DEN) through ten weekly injections in Sprague-Dawley rats or a single injection in C57Bl/6 mice. Yu *et al.* found that gut sterilization and TLR4 depletion decreased HCC tumor incidence and growth and concluded that LPS accumulation was a promoter of HCC in DEN-treated rodents [3].

The importance of the LPS-TLR4 pathway in hepatocarcinogenesis has recently been more firmly established by a very extensive study by Dapito *et al.* [4]. In that study, HCC was induced in several genetically different mouse lines following protocols consisting in an initial injection of DEN followed by a choline-deficient diet or multiple carbon tetrachloride (CCl_4) injections or thioacetamide injections. Dapito *et al.* found that inactivating TLR4 had no effect on HCC tumor incidence, but significantly reduced tumor number and size [4]. A HCC slowdown was observed after DEN/CCl_4 intoxication in wild type mice, whose gut was germ-free or had been sterilized with antibiotics. By contrast, continuous administration of low doses of LPS increased tumor number and size in conventional wild type mice intoxicated with DEN/CCl_4 [4]. Dapito *et al.* concluded that the gut microbiota and LPS-TLR4 pathway play a role in HCC promotion in chronically injured livers. On the other hand, they found no effect of the LPS-TLR4 pathway on HCC initiation, at variance with Yu *et al.* conclusions. Beyond the differences in experimental methods (mouse lines, animal care, and liver injury) between the two studies, this discrepancy could point to other unrecognized gut PAMPs, in addition to LPS, contributing to carcinogenesis in inflammatory livers.

From the standpoint of molecular and cellular mechanisms, an important finding of Dapito *et al.* is that the tumor-promoting effect of TLR4 inflammatory signaling originates from resident liver cells (hepatic stellate cells and hepatocytes) and not bone-marrow-derived cells such as macrophages [4]. NF-κB p65 nuclear translocation was found in hepatic stellate cells and hepatocytes, suggesting that both cell types play a role in the promotion of

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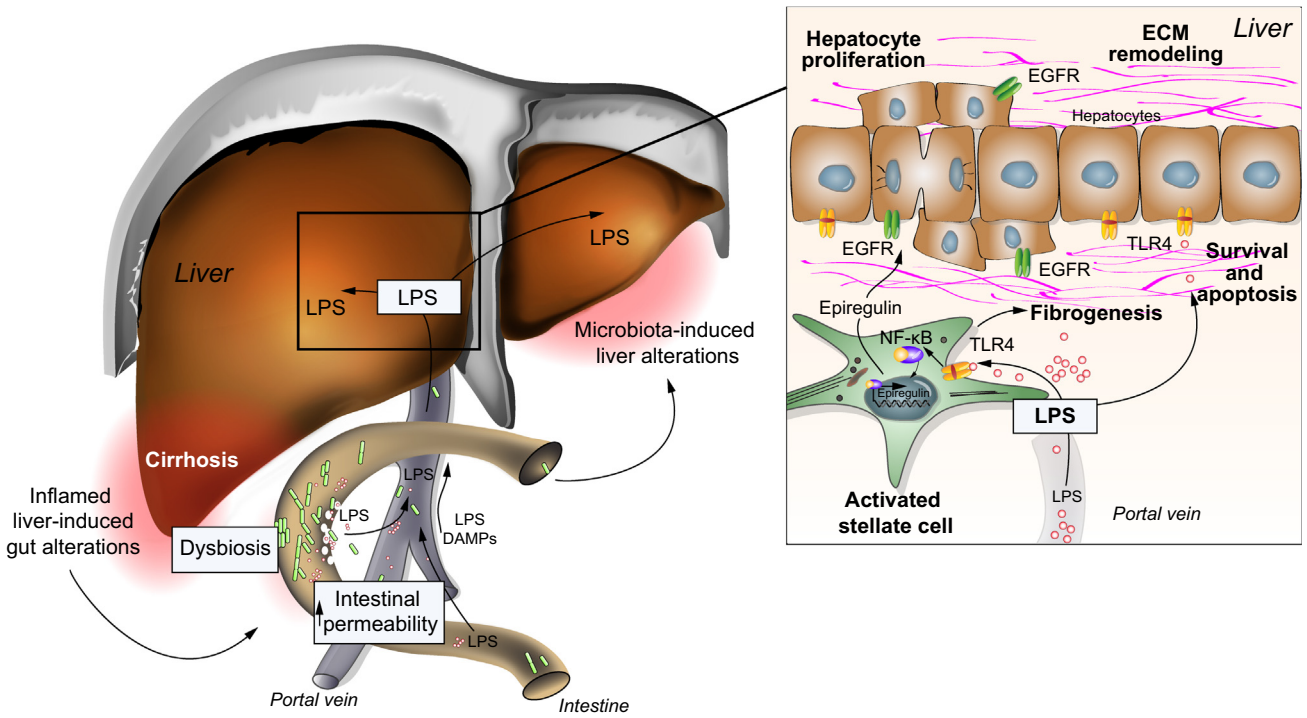

Fig. 1. Hypothetic diagram of HCC promotion by the LPS/TLR4 pathway. The progression of HCC depends on the balance between TRL4-induced proliferative, pro-survival, and apoptotic signals in chronically injured hepatocytes. The chronic inflamed liver is associated with intestinal dysbiosis, gut permeability changes, and PAMP (LPS) translocation to the liver. The TLR4 signaling is activated by LPS in hepatic stellate cells and hepatocytes, resulting in extracellular matrix (ECM) remodeling, fibrogenesis, and secretion of the epiregulin growth factor, which triggers tumor hepatocyte proliferation. The inflammatory liver signals to the gut, maintaining, or amplifying, intestinal dysbiosis.

DEN/CCl_4-induced HCC. These results differ from those of Yu *et al.*, and others, who found that Küpffer cells were the main target of LPS/TLR4 signals, leading to TNFα- and IL6-dependent hepatocyte compensatory proliferation and reduction of oxidative and apoptotic stress [3]. The fact that different authors identify different cell targets for LPS in injured livers is probably not self-contradictory. It may simply point to the complexity of the intercellular dialog between different chronically injured liver cell types aroused by inflammatory signals originating from gut microbiota and eventually leading to HCC. In addition, Dapito *et al.* reported that the LPS/TLR4 pathway upregulates the epiregulin hepatomitogen, an epidermal growth factor (EGF) family member leading to EGFR and HER2 activation during the first stages of DEN/CCl_4 carcinogenesis, whereas it reduces hepatocyte apoptosis by NF-κB nuclear translocation during the late stages of hepatocarcinogenesis [4] (Fig. 1).

Other studies showed that acute [5] and chronic [6,7] liver diseases had also an impact on gut homeostasis and led to dysbiosis and gut barrier permeability, exacerbating viral and chemically induced HCC incidence via NFκB pathway activation [8,9]. Dapito *et al.* suggested that antibiotic-induced gut sterilization could prevent HCC in patients with chronic liver injury [4]. Such a preventive management of cirrhotic patients would require a life-time administration of antibiotic. Counteracting side effects of dysbiosis by probiotic administration or bacteria infusion might be an alternative to antibiotic therapy. In fact, approaches based on gut microbiota manipulation have already been evaluated for other dysbiosis-associated disorders. Vrieze *et al.* performed a double-blind randomized control trial, which showed that bacterial transplantation by lean-donor feces infusion in patients with metabolic syndrome led to improvement of hepatic and peripheral insulin resistance [10]. It would be interesting to evaluate this type of clinical setting in patients at high risk to develop HCC.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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